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Olanzapine-induced changes in glucose metabolism are independent of the melanin-concentrating hormone system

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Abstract Atypical antipsychotic drugs such as Olanzapine (Ola) induce weight gain and metabolic changes associated with the development of type 2 diabetes. The mechanisms underlying these undesired side-effects are currently unknown. Chagnon et al. showed that the common allele rs7973796 of the prepro-melanin-concentrating hormone (PMCH) gene is associated with a greater body mass index in Ola-treated schizophrenic patients. As PMCH encodes for the orexigenic neuropeptide melanin-concentrating hormone (MCH), it was hypothesized that MCH is involved in Ola-induced metabolic changes. We have recently reported that the intragastric infusion of Ola results in hyperglycaemia and insulin resistance in male rats. In order to test in vivo the possible involvement of the PMCH gene in the pathogenesis of Ola side-effects, we administered Ola intragastrically in wild-type (WT) and PMCH knock-out (KO) rats. Our results show that glucose and corticosterone levels, as well as endogenous glucose production, are elevated by the infusion of Ola in both WT and KO animals. Thus, the lack of MCH does not seem to affect the acute effects of Ola on glucose metabolism. On the other hand, these effects might be obliterated by compensatory changes in other hypothalamic systems. In addition, possible modulatory effects of the MCH KO on the long term effects of Ola, i.e. increased adiposity, body weight gain, have not been investigated yet.

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1. Introduction

Treatment with atypical antipsychotic drugs is associated with significant weight gain and metabolic disturbances including hyperglycaemia and insulin resistance. Olanzapine (Ola) is one of the atypical antipsychotic drugs inducing the most dramatic weight gain (Sacher et al., 2008). The mechanisms underlying the metabolic changes induced by Ola are still far from clear. The susceptibility to body weight changes in psychotic patients due to atypical antipsychotics has been hypothesized to partly be of genetic origin. Chagnon et al. in 2004 used a linkage analysis in 21 families with schizophrenia or bipolar disorders (508 family members) and showed linkage of the chromosomal region 12q24 with the phenotype of obesity under the use of antipsychotics. This region is located at less than 1 centimorgan from the prepro-melanin-concentrating hormone (PMCH) gene, encoding the orexigenic neuropeptide melanin-concentrating hormone (MCH) (Chagnon et al., 2004). Later studies by the same authors in unrelated patients showed that the common allele rs7973796 of PMCH gene is associated with a greater body mass index in Ola-treated patients (Chagnon et al., 2007). Thus it was hypothesized that Ola may stimulate PMCH expression and release, and thereby the development of obesity (Chagnon et al., 2007).

MCH is expressed in the lateral hypothalamus and the zona incerta (Bittencourt et al., 1992; Sita et al., 2007), areas involved in the regulation of eating behaviour and energy homeostasis (Qu et al., 1996). MCH neurons project broadly throughout the central nervous system, suggesting that MCH may function as a neurotransmitter and/or a neuromodulator to regulate behavioural functions (Bittencourt et al., 1992; Skofitsch et al., 1985). Processing of PMCH results in the production of 3 neuropeptides: neuropeptide glycine-glutamic acid (N-GE), neuropeptide glutamic acid-isoleucine (N-EI) and MCH (Saito and Maruyama, 2006). When injected centrally in rats, MCH stimulates food intake (Qu et al., 1996). Two receptors are known in humans: MCH receptor-1 (MCHR1) and -2 (Sailer et al., 2001), while only MCHR1 was identified in rodents. Antagonism of this receptor in rodents leads to a decreased food intake and weight gain (Shearman et al., 2003). MCHR1-deficient mice have normal bodyweight but are hyperphagic and hyperactive (Marsh et al., 2002). PMCH knock-out (KO) mice showed reduced body weight and an increased metabolic rate (Shimada et al., 1998). Conversely mice over-expressing PMCH are obese and insulin resistant (Ludwig et al., 2001).

We have shown earlier that intragastric Ola induces hyperglycaemia and insulin resistance (Girault et al., 2012). In the present study, we determined whether the metabolic side-effects of Ola persist in rats in the absence of the MCH system using the PMCH KO rat model (Smits et al., 2006). We acutely administered Ola intragastrically to PMCH KO and wild-type (WT) rats to investigate the role of MCH in the pathogenesis of the metabolic side-effects of Ola.

2. Materials and methods

2.1. Ethic statement

All experiments were approved by the animal care committee of the Royal Netherlands Academy of Arts and Sciences.

2.2. Animals

The PMCH KO rat ($Pmch^{1Hubr}$) was generated by target-selected ENU-driven mutagenesis (Smits et al., 2006). Briefly, high-throughput re-sequencing of genomic target sequences in progeny from mutagenized rats revealed an ENU-induced premature stop codon in exon 1 (K50X) of PMCH gene in a rat (Wistar/Crl background). The heterozygous mutant animal was outcrossed to WT Hsd Wistar background for six generations to eliminate confounding effects from background mutations induced by ENU. Further details regarding the selection of the animals can be found in Mul et al., 2010. WT littermates (with similar genetic backgrounds) were used as controls. PMCH KO rats were viable into adulthood and fertile and appeared phenotypically normal despite their lower body weight. Two rats were housed together until the surgery, under controlled experimental conditions (12:12-h light-dark cycle, light period 06:00–18:00, 21 ± 1 °C, 60% relative humidity). The standard fed diet (semi-high-protein chow: RM3, 26.9% crude protein, 11.5% fat, and 61.6% carbohydrates; 3.33 kcal/g AFE; SDS, Witham, UK) was provided ad libitum together with water. Only male rats were used in the present study.

2.3. Genotyping

Genotyping was done using the KASPar SNP Genotyping System (KBiosciences, Hoddesdon, UK; as described in (van et al., 2008) using gene-specific primers (forward common: TTAAT ACATT CAGGA TGGGG AAAGC CTTT; reverse wild type: GAAGG TGACC AAGTT CATGCT CGATC TTTCT GCGGT ATCTT CCTT; and reverse homozygous: GAAGG TCGGA GTCAA CGGAT TCGAT CTTTC TGCGG TATCT TCCTA). All pups were genotyped at 3 weeks of age. Genotypes were reconfirmed when experimental procedures were completed.

2.4. Drugs

The dose of Ola chosen in the present study was identical to the effective dose used in our previous experiments (Girault et al., 2012) and selected to parallel the clinical setting based on 70% dopamine D2 receptor occupancy, which represents a threshold in humans associated with optimal clinical response (Kapur et al., 2003). The route of administration was chosen such that a continuous infusion of freshly made solution was possible in freely moving, undisturbed animals. Using a surgically implanted intragastric catheter, animals were treated with a primed 36 mg/kg/h infusion during 5 min followed by a continuous 3 mg/kg/h infusion for 160 min (i.e., in total 3.66 mg/rat) of Ola (ChemPacific Corporation, Maryland) dissolved in acidified MilliQ water (pH = 6). Ola solution was prepared in MilliQ water acidified with HCl (1 M) and then brought back to pH 6 using NaOH (1 M).

2.5. Surgical procedure

Animals were anesthetized by an intramuscular injection of 0.6 ml/kg Hypnorm (Janssen, High Wycombe, Buckinghamshire, UK) and a subcutaneous injection of 0.15 ml/kg Dormicum (Roche, Almere, The Netherlands). Silicon catheters were placed into the right jugular vein and the left carotid